

## WHAT IS CLAIMED IS:

- Sub  
A1
- 5
1. A method for monitoring the amplification of a nucleic acid sequence, comprising the steps of:
- providing a target nucleic acid sequence;
- providing a first polynucleotide sequence having at least one donor chromophore, the first polynucleotide sequence being complementary to at least a portion of the target nucleic acid sequence;
- providing a second polynucleotide sequence having at least one
- 10 acceptor chromophore, the second polynucleotide sequence being complementary to a least a portion of the target sequence;
- performing polymerase chain reaction to amplify the target nucleic acid sequence;
- hybridizing the first and second polynucleotide sequences to the
- 15 target nucleic acid sequence, such that when the first polynucleotide sequence and the second polynucleotide sequence are hybridized to the target nucleic acid sequence, the donor chromophore and acceptor chromophore are in an energy transfer relationship; and
- irradiating the mixture to detect hybridizations of the first and second
- 20 polynucleotide sequences by fluorescence energy transfer from the one or more donor chromophores of the first polynucleotide sequence to the one or more acceptor chromophores of the second polynucleotide sequence.
2. The method of claim 1, wherein the reaction mixture is a homogeneous format.
- 25 3. The method of claim 1, wherein the target nucleic acid comprises double stranded or denatured DNA.
4. The method of claim 1, wherein the amplification is monitored for each PCR cycle.
5. The method of claim 1, wherein the target nucleic acid sequence
- 30 comprises RNA.
- 09997374-112301

6. The method of claim 1, wherein the target nucleic acid sequence comprises synthetic polynucleotide.

7. The method of claim 1, wherein the hybridization and amplification reactions occur entirely in solution.

5 8. The method of claim 1, wherein at least one of the first polynucleotide, the second polynucleotide, or the target nucleic acid is bound to a solid support or matrix that is insoluble in the analyte solution.

9. The method of claim 8, wherein the solid support or matrix is selected from the group consisting of glass, metals, silicon, organic polymers,  
10 membranes, and biopolymers.

10. The method of claim 1, wherein the polynucleotide sequence comprises a plurality of donor chromophores.

11. The method of claim 1, wherein the polynucleotide sequence comprises a plurality of acceptor chromophores.

15 12. The method of claim 1, wherein the step of hybridizing the first and second polynucleotide sequences to the target nucleic acid sequence takes place during a PCR cycle.

13. The method of claim 1, wherein the step of irradiating the mixture to detect hybridizations of the first and second polynucleotide sequences by  
20 fluorescence energy transfer from the one or more donor chromophores of the first polynucleotide sequence to the one or more acceptor chromophores of the second polynucleotide sequence takes place during a PCR cycle.

14. The method of claim 1, wherein each of the donor chromophores and the acceptor chromophores is selected from the group consisting of 4,4'-  
25 Diisothiocyanatodihydro-stilbene-2,2'-disulfonic acid, 4-acetamido-4'-isothiocyanato-stilbene-2,2'-disulfonic acid, 4,4'-Diisothiocyanatostilbene-2,2'-disulfonic acid, Succinimidyl pyrene butyrate, Acridine isothiocyanate, 4-

00997374-12501  
10927-47655

15. The method of claim 1, wherein the step of providing a target nucleic acid sequence further comprises the steps of providing an mRNA sequence producing a double-stranded cDNA sequence from the mRNA sequence.

16. A method for monitoring the amplification of a nucleic acid sequence, comprising the steps of:

providing a target nucleic acid sequence;

5 providing a first polynucleotide sequence having at least one donor chromophore, the first polynucleotide sequence being complementary to at least a portion of the target nucleic acid sequence;

providing a second polynucleotide sequence having at least one acceptor chromophore, the second polynucleotide sequence being complementary to a least a portion of the target sequence;

10 providing four different nucleoside triphosphates, a thermostable amplification enzyme, and two primers, wherein the primers are substantially complementary to the target polynucleotide sequence;

denaturing the target nucleic acid sequence to form single stranded nucleic acids at an appropriate temperature;

15 hybridizing the first and second polynucleotide sequences to the target nucleic acid sequence, such that when the first polynucleotide sequence and the second polynucleotide sequence are hybridized to the target nucleic acid sequence, the donor chromophore and acceptor chromophore are in an energy transfer relationship; and

20 irradiating the mixture to detect hybridizations of the first and second polynucleotide sequences by fluorescence energy transfer from the one or more donor chromophores of the first polynucleotide sequence to the one or more acceptor chromophores of the second polynucleotide sequence;

25 elongating of the target polynucleotide sequence under conditions that the target polynucleotide sequence is amplifiable.

17. The method of claim 15, wherein the target nucleic acid comprises double stranded DNA.

18. The method of claim 15, wherein the target nucleic acid sequence comprises synthetic polynucleotide.

Sub  
A2

09997374-1-3331

19. The method of claim 16, wherein the hybridization and amplification reactions occur entirely in solution.

20. The method of claim 16, wherein at least one of the first polynucleotide, the second polynucleotide, or the target nucleic acid is bound to a solid support or matrix that is insoluble in the analyte solution.

21. The method of claim 20, wherein the solid support or matrix is selected from the group consisting of glass, metals, silicon, organic polymers, membranes, and biopolymers.

22. The method of claim 16, wherein the polynucleotide sequence comprises a plurality of donor chromophores.

23. The method of claim 16, wherein the polynucleotide sequence comprises a plurality of acceptor chromophores.

24. The method of claim 16, wherein the step of providing a target nucleic acid sequence further comprises the steps of providing an mRNA sequence producing a double-stranded cDNA sequence from the mRNA sequence.